(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 470 452 A2**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **91112558.1**

(51) Int. Cl.5: **C08B 37/16**, A61K 47/40

(22) Date of filing: **26.07.91**

(30) Priority: **09.08.90 IT 2125790**

(43) Date of publication of application:
12.02.92 Bulletin 92/07

(84) Designated Contracting States:
AT BE CH DE ES FR GB IT LI LU NL

(71) Applicant: **STAROIL LIMITED**
No. 4 Columbus Centre Wickhams Cay Road Town
British Virgin Islands(GB)

(72) Inventor: **Bruzzese, Tiberio**
Via Frua, 21/6
I-20146 Milano(IT)
Inventor: **Mozzi, Giovanni**
Piazzale Loreto, 9
I-20131 Milan(IT)

(74) Representative: **Luksch, Giorgio, Dr.-Ing. et al**
Ing. A. Giambrocono & C. S.r.l. Via Rosolino Pilo, 19/b
I-20129 Milano(IT)

(54) **A method for the production of complexes of long chain polyunsaturated fatty acids and their derivatives, with cyclodextrins, and the resulting complexes.**

(57) A method for the production of a complex formed by a long chain polyunsaturated fatty acid, a salt of it, alkyl ester C_1-C_3 or glyceride, or a mixture thereof, with a cyclodextrin, and the resulting complex .

EP 0 470 452 A2

The present invention is concerned with both a method of producing new complexes of long chain polyunsaturated fatty acids, their salts and esters, inclusive of fish and vegetable oil glycerides, with cyclodextrins (α-, β- and γ -cyclodextrin and hydroxypropyl-β-cyclodextrin), and the resulting new complexes.

The dietetic and pharmaceutical use of fish oils and of polyunsaturated fatty acids, therein contained in the form of glycerides, is extending on the ground of their claimed fat lowering (Y. Tamura et al., Prog. Lipid. Res., 25, 461, 1986; C. Von Schacky, Ann. Intern. Med., 107, 890, 1987) and platelet anticoagulant properties (C.R.M. Hay et al., Lancet ii, 1269, 1982), highly interesting in the prevention and treatment of the most important cardiovascular diseases. At the same time a most pressing evidence of other therapeutic uses is arising in the treatment of psoriasis, rheumatoid arthritis (J.M. Kremer et al., Ann. Intern. Med., 106, 497, 1987; R.T. Sperling et al., Arth. Rheum., 30, 988, 1987), polyposis of the colon , arterial hypertension (K.M. Bonaa et al., N. Engl. J. Med., 322, 795, 1990; M.R. Knapp et al., N. Engl. J. Med., 320, 1037, 1989) and in the therapy of cancer.

The fish oils, some vegetable oils, the polyunsaturated fatty acids making them up and their derivatives exhibit however some characteristics markedly limiting their use. They are liquid at room temperature, unctuous, with an unpleasant taste and odour and easily oxidized in the air owing to the large number of carbon-carbon double bonds in their molecules, with consequent deterioration in time of the organoleptic characteristics and potential formation of epoxy groups considered as having toxic effects. These characteristics markedly curtail the dietetic and pharmaceutical use of these products. Dosage forms such as tablets are for instance precluded as are hard gelatin capsules, syrups, drinking vials, water-dispersible granules in single dose sachets.

In practice the sole pharmaceutical form used is the soft gelatin capsules, normally of considerable size owing to the rather high dosage (up to several grams daily). This extra large size makes the use of the capsules by the elderly quite difficult, who are the principal users owing to the pathologies mentioned above and who would have no difficulty if they were to take e.g., syrups or water dispersible granules.

To forestall these disadvantages U.S. Patent no. 4 438 106 describes inclusion compounds of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), their alkaline salts and alkyl esters in cyclodextrin, obtained by a complexation reaction occurring in the presence of large quantities of a polar organic solvent and through boiling. In the resulting complexes the quantity of oleaginous substance is not more than 15% by weight at the best of times. A like concentration of active substance requires that frequent daily doses be administered in order to attain the pharmacological active quantity of active ingredient; moreover oversize dosage units will have to be prepared to ensure such quantities, not to mention the excessive use of the complexant cyclodextrin, inactive per se but with a negative influence as regards the cost of the finished product. Moreover, the known process calls for the use of large quantities of solvents negatively influencing the production costs and entailing rather considerable risks to the operator (fire, explosion, intoxication by inhalation) and to the user (unavoidable traces of solvents in the finished product).

It was surprisingly discovered that a process requiring no organic solvents permitted the production of complexes of long chain polyunsaturated fatty acids, their salts and esters, inclusive of fish and vegetable oil glycerides, with cyclodextrins (α -, β- and γ-cyclodextrin and hydroxypropyl-β-cyclodextrin) having a content by weight of oleaginous substance significantly higher than the limits of the known art as mentioned above, with consequent avoidance of the before mentioned disadvantages.

The method of the invention comprises: solution of cyclodextrin in simple, preferably distilled, water, introduction of the active oleaginous substance in the resulting solution thereby obtaining an heterogenic mixture, submission of the mixture to stirring for from about 1 to about 24 hours at a temperature comprised between 0 and 100 °C, preferably at room temperature, from which the desired complex precipitates in a solid, crystalline form; the complex is recovered by filtration thereafter whased and dried.

The phase of washing the invention complex is carried out with water or with organic solvent such as methanol, ethanol, etc., or their mixture. Drying may be done in oven at a moderate temperature.

The resulting products are characterized by the fact that the concentration of oleaginous substance present in the complex is higher than 18% by weight, preferably from about 20% to about 50% by weight.

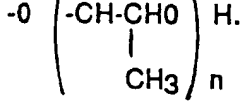
Complexation is performed of various types of fish oils, vegetable oils and long chain polyunsaturated fatty acids, their salts and alkyl and glyceryl esters, with cyclodextrins in varying degrees of polymerization and substitution.

By way of example, natural fish oils can be used (mackerel, trout, herring, sardines, tuna fish, salmon , cod, etc.) or purified fish oils, or even oils concentrated by known techniques in glycerides containing polyunsaturated acids having high molecular weight. Starting from the acids themselves

analogous complexes were prepared in a free or variously salified form, or from the corresponding C_1-C_3 esters (preferably from ethyl esters), or from the relative glycerides obtained through synthesis.

Preference is given to the use of ω-3 polyunsaturated fatty acids in that considered the pharmacologically most active constituents of fish oils, and the ω-6 fatty acids, typical of some vegetable oils. Of the ω-3 polyunsaturated fatty acids, the long chain ones (C_{20}-C_{22}) , more precisely cis-5,8,11,14,17-eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) and their derivatives, in particular the ethylesters, are considered most interesting. Of the ω-6 fatty acids, preference is given to γ-linolenic acid (C_{18}) and its derivatives.

All the above mentioned compounds can be present in the oils used in the complexation, either alone, as a single component, or as a mixture of them in a varying ratio. The cyclodextrins that can be used in complexation may be either α-, β- or γ-cyclodextrin, characterized by 6, 7 or 8 D-glucopyranoside units forming a cyclic cavity. Hydroxypropyl-β-cyclodextrin can also be used; it is derived from β-cyclodextrin through total or partial replacement of the hydroxyls of the ring-forming glucoside units with an $-O-CH_2-CHOH-CH_3$ radical or one of its polymers



Unlike the oleaginous substances used in the preparation, the new complexes appear as gliding, not unctuous, nearly odourless and tasteless powders and can therefore be used to prepare a variety of pharmaceutical forms such as tablets, hard gelatin capsules , syrups, drinking vials, water dispersible granules in sachets etc. Furthermore, they maintain in time their satisfactory initial organoleptic and chemical characteristics, since their stability is better than the starting oils.

In this context the term 'complexes' specially means inclusion compounds of long chain polyunsaturated substances enclosed in the hydrophobic cavities of the cyclodextrins.

The following examples are solely given to better illustrate the invention, but in no way are they to be intended as limiting the scopes of the invention itself.

The oil present in the complex was determined by performing the U.V. absorption spectrum at 195 to 300 nm of an ethanol solution of it and then comparing the maximum absorbance with that of an analogous standard solution.

The gas-chromatographic determination (G.C.) of the oil present in the complex was performed on a chloroform extract of it under the following operative conditions:

- internal standard: 0.003% methyl palmitate
- column : SBT ^{TB}-1, 15 m, 0.25 mm int. dia., 0.25 μ thickness
- injection port: PTV
- splitting: 1:70
- column temp. programmed at from 160 to 240 $^{\circ}$C
- carrier gas: helium
- adjuvant gas: nitrogen
- detector: F.I.D.

The yields were obtained by calculating the percent ratio between product obtained and sum of oil plus starting cyclodextrin.

The cyclodextrins used contained varying quantities of water (up to 12%). In the following examples the percent assay values of the cyclodextrins used are shown in brackets.

Example 1

Dissolve 9.5 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 100 ml of distilled water at 60 $^{\circ}$C. At a temperature of 55 $^{\circ}$C and while stirring add 6.5 g of oleaginous substance containing DHA ethylester (assay value $\geq$ 80%). Sonicate, allow to stand for 1 h at 45 $^{\circ}$C then homogenize with ULTRA-TURRAX. Filter, wash the solid residue with 20 ml of methanol and dry it in an oven at 45 $^{\circ}$C.

The product obtained contains 27% of oil (UV assay value calculated on substance as such) equivalent to a molar ratio to β-cyclodextrin of about 1.25 : 1.

Example 2

Dissolve 18.1 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 500 ml of distilled water at 40-45 $^{\circ}$C. Cool to room temperature and while stirring add 5 g of oleaginous substance containing DHA ethylester (assay value $\geq$ 80%). Cool immediately to 2 $^{\circ}$C and maintain under stirring at this temperature for 7 h. Filter, wash with about 50 ml of distilled water and dry in oven at 45 $^{\circ}$C.

The product obtained with a yield of about 85%, contains 23.7% of oil (G.C. assay value on the dry product) equivalent to a molar ratio to β-cyclodextrin of about 1:1.

Example 3

Dissolve 18.1 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in

500 ml of distilled water at 40-45 °C. Cool to room temperature and add 10 g of oleaginous substance containing DHA ethylester (assay value ≥80%). Agitate on shaker for 22 h at 26 °C. Filter, wash with about 50 ml of distilled water and dry in an oven at 45 °C.

The product, obtained with a yield of about 93%, contains 38.5% of oil (G.C. assay value on the dry product) equivalent to a molar ratio to β-cyclodextrin of about 2 : 1.

Example 4

Dissolve 38 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 1 litre of distilled water at 40-45 °C. Cool to room temperature and while stirring add 20 g of oleaginous substance containing EPA and DHA ethylesters (combined assay value ≥ 80%). Maintain under stirring at room temperature for 7 h. Filter, wash with about 100 ml of distilled water and dry in an oven at 45 °C.

The product, obtained with a yield of about 90%, contains 36.6% of oil (G.C. assay value on the dry product), equivalent to a molar ratio to β-cyclodextrin of about 2:1.

Example 5

Dissolve 19 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 500 ml of distilled water at 40-45 °C. Cool to room temperature and while stirring add 17.3 g of oleaginous substance containing EPA and DHA ethylesters (combined assay value ≥ 80%). Maintain under stirring at room temperature for 7 h. Filter, wash with about 50 ml of distilled water and dry in an oven at 45 °C.

The product, obtained with a yield of about 80%, contains 44.4% of oil (G.C. assay value on the dry product), equivalent to a molar ratio to β-cyclodextrin of about 3:1.

Example 6

Dissolve 3.2 g of α-cyclodextrin (assay value about 90% calculated on the substance as such) in 20 ml of distilled water and while stirring add 1 g of oleaginous substance containing EPA and DHA ethylesters (combined assay value ≥ 80%). Maintain under stirring at room temperature for 5 h. Filter, wash with about 10 ml of distilled water and dry in an oven at 45 °C.

The product, obtained with a yield of about 96%, contains 26.3% of oil (G.C. assay value on the dry product), equivalent to a molar ratio to α-cyclodextrin of about 1:1.

Example 7

Dissolve 4.2 g of γ-cyclodextrin (assay value about 90% calculated on the substance as such) in 20 ml of distilled water and while stirring add 1 g of oleaginous subtance containing EPA and DHA ethylesters (combined assay value ≥ 80%). Maintain under stirring at room temperature for 5 h. Filter, wash with about 10 ml of distilled water and dry in an oven at 45 °C.

The product, obtained with a yield of about 96%, contains 20.3% of oil (G.C. assay value on the dry product), equivalent to a molar ratio to γ-cyclodextrin of about 1:1.

Example 8

Dissolve 3.9 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 100 ml of distilled water at 40-45 °C. Cool to room temperature and while stirring add 1 g of oleaginous substance containing EPA and DHA triglyceride esters (combined assay value > 50%). Maintain under stirring at room temperature for 4.5 h. Filter, wash with about 20 ml of distilled water and dry in an oven at 45 °C.

The product, obtained with a yield of about 80%, contains 22.2% of oil (U.V. assay value on the dry product), equivalent to a molar ratio of the triglyceride acid radical to β-cyclodextrin of about 1:1.

Example 9

Dissolve 3.4 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 50 ml of distilled water at 40-45 °C. Cool to room temperature and dissolve 1 g of DHA sodium salt. Maintain under stirring at room temperature for 3 h and evaporate the solution to dryness under reduced pressure then wash the residue with 5 ml of distilled water, filter and dry in an oven at 45 °C. The product obtained contains 20.6% of DHA sodium salt (U.V. assay value on the dry product), equivalent to a molar ratio to β-cyclodextrin of about 1:1.25.

Example 10

Dissolve 4.2 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 100 ml of distilled water at 40-45 °C. Cool to room temperature and while stirring add 1 g of an oleaginous substance containing EPA acid (assay value ≥ 80%). Maintain under stirring at room temperature for 5.5 h. Filter, wash with about 20 ml of distilled water and dry in an oven at 45 °C.

The product obtained contains 19.6% of oil

(U.V. assay value on the dry product), equivalent to a molar ratio to β-cyclodextrin of about 1:1.

Example 11

Dissolve 4.6 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 100 ml of distilled water at 45-50° C. Cool to room temperature and while stirring add 1 g of an oleaginous substance containing γ-linolenic acid (assay value ≥ 85%). Maintain under stirring at room temperature for 4.5 h. Filter, wash with about 20 ml of distilled water and dry in an oven at 45° C.

The product, obtained with a yield of about 80%, contains 19.5% of oil (U.V. assay value on the dry product), equivalent to a molar ratio to β-cyclodextrin of about 1:1.

Example 12

Dissolve 3.9 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 100 ml of distilled water at 40-45° C. Cool to room temperature and add 2 g of oleaginous substance containing EPA ethylester (assay value ≥ 80%). Agitate on a shaker for 22 h at 26° C. Filter, wash with about 20 ml of distilled water and dry in an oven at 45° C.

The product, obtained with a yield of about 90%, contains 35.7% of oil (U.V. assay value on the dry product), equivalent to a molar ratio to β-cyclodextrin of about 2:1.

Example 13

Dissolve 4.2 g of β-cyclodextrin (assay value about 88% calculated on the substance as such) in 100 ml of distilled water at 45-50° C. Cool to room temperature and while stirring add 1 g of oleaginous substance containing γ-linolenic acid ethylester (assay value ≥ 80%). Maintain under stirring at room temperature for 4.5 h. Filter, wash with about 20 ml of distilled water and dry in an oven at 45° C.

The product, obtained with a yield of about 85%, contains 21.4% of oil (U.V. assay value on the dry product), equivalent to a molar ratio to β-cyclodextrin of about 1:1.

Claims

1. A method of producing a complex containing at least a long chain polyunsaturated fatty acid or a derivative of it, and cyclodextrin, comprising dissolution of cyclodextrin in water, addition of the active oleaginous substance to the resulting solution, in order to form a heterogeneous mixture which will be submitted to stirring for a period of 1 to 24 h at a temperature of between 0° and 100° C, and from which thereafter the desired complex precipitates in the form of a crystalline solid recovered by filtration, washing and drying.

2. A method in accordance with claim 1 in which cyclodextrin is dissolved in distilled water.

3. A method in accordance with claims 1 and 2 in which the temperature at which the heterogeneous mixture is submitted to stirring is the room temperature.

4. A method in accordance with the foregoing claims in which cyclodextrin is selected from the group comprising α-, β-, γ-cyclodextrin and hydroxypropyl-β-cyclodextrin.

5. A method in accordance with the foregoing claims in which the fatty acid is selected from the group comprising EPA, DHA and γ-linolenic acid.

6. A method in accordance with the foregoing claims in which washing of the filtered complex is performed with water or with an organic solvent or with a mixture thereof.

7. A complex formed by at least a long chain polyunsaturated fatty acid, by a salt of it, by a C_1-C_3 alkyl or glyceril ester of it, with a cyclodextrin, characterized by the fact that the active oleaginous substance is present in the complex at a concentration higher than 18% by weight.

8. A complex in accordance with claim 7, in which the active oleaginous substance is present at a concentration of from about 20% to about 50% by weight.

9. A complex in accordance with claims 7 or 8 in which the polyunsaturated fatty acid belongs to the ω-3 and ω-6 series.

10. A complex in accordance with claims 7 or 8 in which the polyunsaturated fatty acid has a chain of 18-22 carbon atoms.

11. A complex in accordance with claim 9 in which the ω-3 series acid is selected from the group comprising EPA and DHA.

12. A complex in accordance with claim 9 in which the ω-6 series acid is γ-linolenic acid.

13. A complex in accordance with claims 7 or 8 in

which the alkyl ester derivatives are ethylesters.

14. A complex in accordance with claims 7 or 8 in which cyclodextrin is selected from the group comprising α -, β-and γ-cyclodextrin and hydroxypropyl-β-cyclodextrin.

15. A complex in accordance with claim 8 that is EPA ethylester and β-cyclodextrin.

16. A complex in accordance with claim 8 that is DHA ethylester and β-cyclodextrin.

17. A complex in accordance with claim 8 that is EPA ethylester + DHA ethylester with β-cyclodextrin.

18. A pharmaceutical formula containing the complex in accordance with claims 7 or 8, with at least a pharmaceutically acceptable excipient.

19. The use of the complex in accordance with claims 7 or 8, for the preparation of a formula intended for dietetic and therapeutic use.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 470 452 A3**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **91112558.1**

(51) Int. Cl.5: **C08B 37/16**, A61K 47/40

(22) Date of filing: **26.07.91**

(30) Priority: **09.08.90 IT 2125790**

(43) Date of publication of application:
12.02.92 Bulletin 92/07

(84) Designated Contracting States:
AT BE CH DE ES FR GB IT LI LU NL

(88) Date of deferred publication of the search report:
29.04.92 Bulletin 92/18

(71) Applicant: **STAROIL LIMITED**
No. 4 Columbus Centre Wickhams Cay Road
Town
British Virgin Islands(GB)

(72) Inventor: **Bruzzese, Tiberio**
Via Frua, 21/6
I-20146 Milano(IT)
Inventor: **Mozzi, Giovanni**
Piazzale Loreto, 9
I-20131 Milan(IT)

(74) Representative: **Luksch, Giorgio, Dr.-Ing. et al**
Ing. A. Giambrocono & C. S.r.l. Via Rosolino Pilo, 19/b
I-20129 Milano(IT)

(54) **A method for the production of complexes of long chain polyunsaturated fatty acids and their derivatives, with cyclodextrins, and the resulting complexes.**

(57) A method for the production of a complex formed by a long chain polyunsaturated fatty acid, a salt of it, alkyl ester C_1-C_3 or glyceride, or a mixture thereof, with a cyclodextrin, and the resulting complex .

EP 0 470 452 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 11 2558

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	FR-A-2 550 445 (KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO) * page 4 * * page 9, line 4 - line 9 * * claims 1-8 *	1-19	C 08 B 37/16 A 61 K 47/40
X	FR-A-2 596 617 (INSTITUT FRANCAIS DE RECHERCHE SCIENTIFIQUE POUR LE DEVELOPPEMENT) * page 2, line 23 - line 31 * * example 1 *	1-17	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)
C 08 B

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	27 February 92	LENSEN H.W.M.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document